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Occurrence of new case-bearer on cereals, *Coleophora perplexella* Toll, 1960 (Lepidoptera: Coleophoridae)

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Abstract

Coleophora perplexella Toll, 1960 it is native of Spain and Portugal, but it has detected out of its origin area, and was found in Sardinia, Morocco and Algeria without its host plant being identified. Recently has been observed in France (Toulon) on *Hordeum murinum* L. and *Bromus sterilis* L. (Poaceae). However, it was never cited among the Tunisian entomofauna. In addition, *C. perplexella* was never known as agricultural pest in its area or elsewhere. Moreover, no deep studies diagnosis and biology were conducted. This paper is a first study on *C. perplexella,* detected as an agricultural pest on oat (*Avena sativa* L., 1753) and barely (*Hordeum vulgare* L., 1753) making serious damages on early seedlings. Besides, a description and illustrations of different biological stage was provided. Results highlight a difference between the appearance dates of emerged new adults under both controlled and ambient conditions. According these results we can conclude on adaptation plasticity to the environmental conditions. In fact, *C. perplexella* can survive and cause damage at temperature between 7-10º C from December to February, and 23-25º C of controlled conditions.
KEY WORDS: Lepidoptera, Coleophoridae, *Coleophora perplexella*, agricultural pest, biology, cereal, invasive species, Tunisia.

La aparición de un nuevo coleofórido sobre cereales, *Coleophora perplexella* Toll, 1960 (Lepidoptera: Coleophoridae)

Resumen

Coleophora perplexella Toll, 1960 es originaria de España y Portugal, pero ha sido detectada fuera de su área original y fue encontrada en Cerdeña y Marruecos sin identificar su planta nutricia. Recientemente ha sido observada en France (Toulon) sobre *Hordeum murinum* L. and *Bromus sterilis* L. (Poaceae). Sin embargo, nunca había sido citada de la entomofauna tunecina. Además, *C. perplexella*, nunca fue conocida como plaga agrícola en su área o en otra parte. Además, ningún estudio diagnóstico fue realizado en profundidad o sobre su biología. Este trabajo es el primer estudio sobre *C. perplexella*, detectado como plaga agrícola sobre avena (*Avena sativa* L., 1753) y cebada (*Hordeum vulgare* L., 1753) causando serios daños sobre las plantas de semillas tempranas. Además, se da una descripción de los diferentes estados biológicos. Lo más interesante del resultado es la aparente diferencia que hay entre la aparición de los adultos tanto controladas como ambientales. De acuerdo con estos resultados Podemos concluir sobre la plasticidad de su adaptación a las condiciones ambientales. A decir verdad, *C. perplexella* puede sobrevivir y causar daños a temperaturas entre los 7-10º C de diciembre a febrero y de 23-25º C de las condiciones controladas.
PALABRAS CLAVE: Lepidoptera, Coleophoridae, *Coleophora perplexella*, plaga agrícola; biología, cereal, especie invasiva, Túnez.

[§] CXXXVII Contribution to the knowledge of Coleophoridae.

Introduction

The Coleophoridae has a worldwide distribution with the large dominance in Old World group, and a great diversity in the West Palearctic region and central Asia. Most of them prefer temperate climate regions, with dry or arid conditions, but they are found also in Australia, and South America. The Coleophoridae species are characterized by a small size, dull coloration, and minor economic importance. Nevertheless, the abundance of literature treating this subfamily, with nearly 1500 references (BALDIZZONE *et al*., 2006) reflected their popularity in Europe. They construct portable cases in which they spent most of their larval life and where the pupation takes place. They are commonly known as case-bearers. The case origin and shapes are much diversified among species. They could be made from host material pieces, silk, or a combination of various substances. However, the case shape is specific for each species. During their larval stage, some species can enlarge progressively the origin case or change it by another larger. Larvae are leaf or seed miners and feed without leaving their case. Most of them feed on dicot host plants, with a preference to the Asteraceae, Fabaceae, Lamiaceae, Caryophyllaceae and Chenopodiaceae family. In the other hand, those feeding on monocots use only the seeds of Juncaceae. In Europe and eastern North America, host plants are relatively known, but there not the same for other regions (BALDIZZONE *et al*., 2006). The later reference counts 1342 species, but this number increased up to be almost 1450 species and description of new species continues. The biology of many European species was described, especially by HERING (1957), SUIRE (1961) and EMMET *et al*., (1996).

The *Coleophora lixella* Zeller 1849 group has a particular biology. Their first host plant belongs always to the Lamiaceae family, where the young larva uses the flower to elaborate its primary case to hibernate in it. After, what the caterpillar changes completely its host plant and feeds on any near Poaceae without any preferential species (BALDIZZONE *et al*., 2014). Therefore, there is actually just one specie, *Coleophora ciconiella* Zeller 1849 (= *C. tritici* Lindeman 1881) that completes all its life cycle on grasses. It can attack cereal crops especially wheat and causes damage (VENTURI, 1949). However, this species is rare due may be to the use of pesticides (PATZAK, 1976).

According to BALDIZZONE *et al*. (2006), there are 57 *Coleophora* species in Tunisia. Studies on *Coleophora* species diagnosis and biology are rare. In fact, most species (18 Species) were detected during the 50s of the last Century. *Coleophora gracilella* Toll 1952 and *Coleophora berbera* Baldizzone 1988 were reported in Nefta and Hammamet respectively (BALDIZZONE, 1988). But, the recent and new species *Coleophora tunisiae* Stübner & Baldizzone, 2007 was collected at Ain Draham and Tabarka zone (STÜBNER, 2007). It's a green metallic species whose biology still unknown, but probably grows on Fabaceae family, similarly to the group of metallic green species to which it belongs.

This paper is about the outbreak *C. perplexella* new microlepidopteran cereal pest in Kef region, located in the North West of Tunisia with some morphological and biological data.

Materials and methods

Collecting sites

Coleophora perplexella larvae specimens were collected in December/2016 from different cereal plots showing relevant symptoms. Oat plots were located in Kef-Est at Oued El-Ain (GPS coordinates: 36º 08.910 N, 008º 43.382 E) and Oued Mallegue (GPS coordinates: 36º 18.714 N, 008º 44.860E) and Barely plots (GPS coordinates: 36º 13.963 N, 008º 48.654E) were at Sidi Harraghi.

Insect density

Density measurements were performed at infested plot using a wooden square of 25 cm^2. The square was placed randomly at each plot, then we counted the number hanged and on ground case-bearers inside the square. Three repetitions were carried out in each plot.

Morphological measurements

Collected insects were divided in two lots, the first one was used for insect rearing and the second one was reserved for morphological identification and measurements. Ten case-bearers at last instar were sacrificed to determine the length of each one: The case length was obtained by simple measurement from the oral to the anal opens. However, caterpillars were extracted from their cases by practicing a longitudinal opening along the case. The measurement was taking out when the caterpillar was in elongated position. The length of emerging adult's wingspan was measured and recorded.

Insect rearing

Collected case-bearers were reared on Rihane barley over twelve one liter pots that each one was infested with ten case-bearers. To avoid the risk of infestation by other insects or the leakage of collected one, each pot was covered with insect proof tissue. The insect culture was maintained under tow rearing conditions: at 24 ± 1° C, 12:12 L:D photoperiod and 60-70% RH and at ambient laboratory condition in Kef Graduate School of Agriculture. Sufficient food for insects was provided by new plants grown in separated pots.

Obtained adults from larval rearing were immediately transferred into a nest box to prepare necessary conditions for mating and securing eggs. The nest box was placed in the same conditions of case-bearers rearing. To ensure adults feeding a small box containing solution honeydew renewed periodically, was placed in the nest box where a small piece of paper and leaves of barley were used to support laid eggs and serve as nutrition source for new hatched larvae.

Statistical analyses

Case-bearers density in different plots was compared using one-way ANOVA followed by Duncan test for univariate-comparison when significant differences were observed at $p<0.05$.

Results and discussion

Symptom's detection

During the 2015/2016 campaign, first symptoms including mines on leaves, plants wilting and death with the appearance of glabrous areas were noticed in December 2015 in Barely plot located at Djebel Eddir (Province of Kef, North-Western of Tunisia). At first, damages were suspected to be caused by worms. However, careful examination of the plot revealed the presence of some unknown insect cases (covers) hanging on leaves and abandoned on soil surface. Therefore, some samples were collected and carried to the laboratory for identification. Unfortunately, no emerged adults were obtained because the farmer has already treated his plot with a systemic insecticide.

The next campaign (2016/2017) we cannot detect the pest in the first area, but the prospection of other plots distant from the first one at least 10 Km demonstrated the dispersion of pest on other plots located in Oued El-Ain, Sidi Haraghi and Oued Mallegue (Provence of Kef). The highest degrees of damages were notified on January/2017 in oat plot that revealed a large bare area (Fig. 1a). Attacked leaves looked like old mines (Fig. 1b). A small round hole appeared on each leave (Fig. 1c). Leaves lost their constituents, fade and died. These symptoms are known to be specific to *Coleophora* sp. attack. However, and up to now, there is no report about the effect of *Coleophora* species on cereals.

Examination of attacked plants highlights the existence of some cases hanging at a few degrees on leaves (Fig.1d) and lodged a microlepidopteran larva (Fig. 3a).

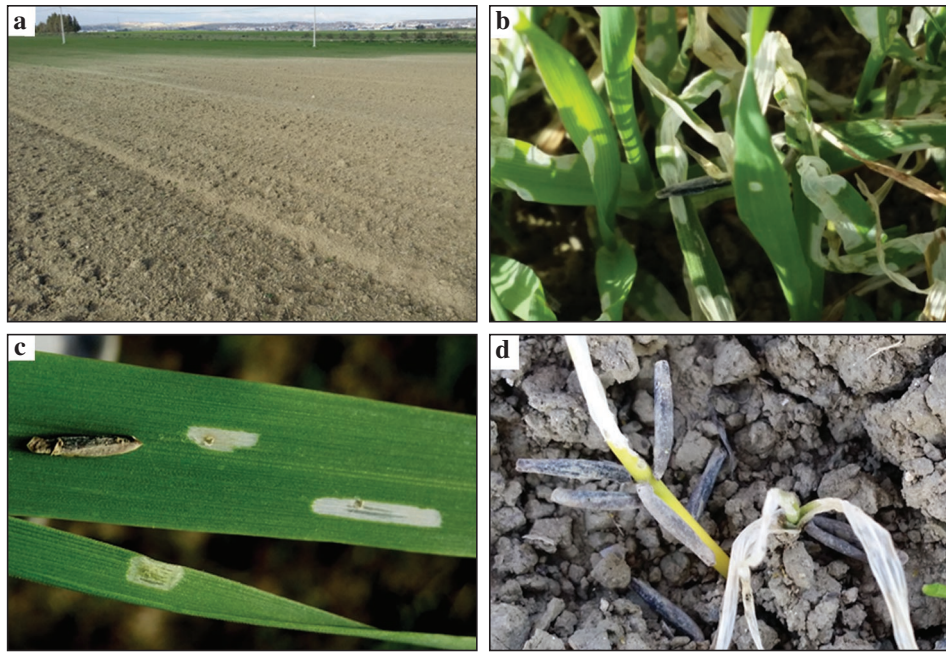

Figure 1.– Damage of *Coleophora perplexella* on oat: **a.** Disappearance of the vegetation covers from oat's plot, due to the pest attack, **b.** Symptoms on oat leaves, **c.** Pest nutrition hole, **d.** Cases hanging on oat leaf.

INSECT DENSITY

Case-bearers density was reported for oat and barely plots located in Oued El-Ain, Oued Mallegue and Sidi Harraghi respectively. Results analyses underline a high case-bearers density (128 case-bearers/m^2) in Oued El-Ain oat plot (Fig. 2). This explains the extinction of all vegetation extended to one Hectare. Vegetation of this area was partially recovered after a treatment with a systemic insecticide, fertilization and abundant irrigation. Concerning oat and barely plots situated in Oued Mallegue and Sidi Harraghi respectively the registered densities are between 90.66 and 85.33 case-bearers/m^2 (Fig. 2). Attacked area highlight a delay in vegetation growth and spike emission. Statistical analysis showed a significant densities difference ($p<0.05$) between oat and barely plots founded in Oued El-Ain and Sidi Harraghi respectively, that's explain the importance of oat plot damage in comparison with the barely one.

Insect description

Larvae: All collected insects were in larvae stage. Each one was protected in a case and thereby named as case-bearer moth (Fig. 3a). This insect belongs to the Lepidoptera order, Gelechioidea suborder, Coleophoridae family and *Coleophora* genus.

Larval case: Is an elongated cylindrical cover with an average length of 7.9 mm and a dark gray color which becomes lighter towards the posterior end with black traits in the anterior part (Fig. 3c). It is characterized by the presence of two openings: one oral and one anal. The oral opening is distinguished by a 45 angle of with respect to a longitudinal axis. This opening provides the larva

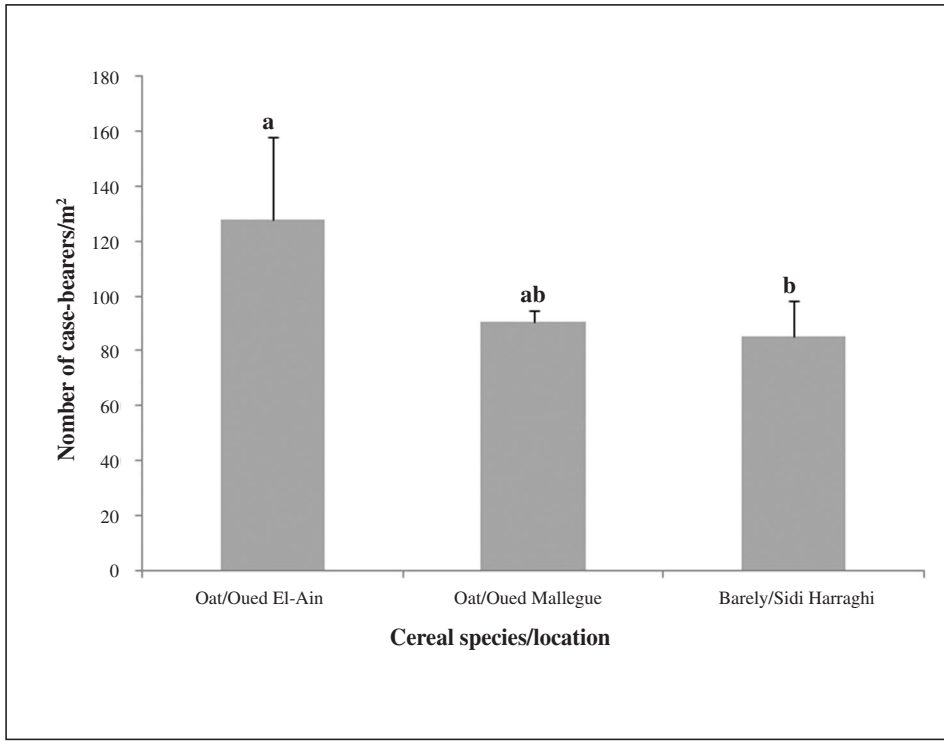

Figure 2.– Case-bearers density in two cereal species and three locations. *The different letters indicate significant differences (P<0.05) according Duncan test.

communication with its environment that will be lined with silk when the larva goes in pupation. While, the anal opening is trilobate, allowing excrements elimination and adult emergence. According to BALDIZZONE (2016), the larval case is entirely made of pleated silk.

Caterpillar: Is illustrated and described by BALDIZZONE (2016). The length measurement of ten caterpillars reported 3.1 mm long with a dark black head, a brown-black thoracic plates and yellow abdominal plates with an exception for the last one (Fig. 3b). In a windy day, caterpillars move the upper leaves and cling to the lower one to control the wind. Pest infestation appears during cold weather (December-February), and can resist due to their cases.

Nymph: Once the caterpillar reaches its last larval stage, it stops feeding and weaves a kind of silk to close the oral end of the case. Pupation undergoes with the case, which makes it difficult to be observed during this stage. However, the release of the nymph from its case showed the presence of a yellow lepidopteran chrysalis, with the appearance of future folded wings, head, eyes, stylus and abdomen (Fig. 3d).

Adults: Were obtained from case-bearers maintained under ambient and controlled conditions [temperature (24±1º C, photoperiod (12:12 L:D) and relative humidity (60-70 %)]. The first emerged adult under controlled conditions was on February/17, 2017. But the maximum emerged adults were registered during March (Table 1). Nevertheless, the first obtained adult under ambient conditions was on April/2017 (Table 1). The adults were characterized by a wingspan length 11.5 mm. The head, thorax and abdomen were yellow. The antennae were long with ocher color. The anterior wings were

yellow with small gray spots and a thin white line along the rib and white ribbed fringes (Fig. 3e). The posterior wings were yellow with white ribbed fringes. The insect identification was carried out by second author basing on case architecture, external morphology and genitalia structures. The insect was identified as *Coleophora perplexella* Toll, 1960. On the basis of current knowledge, this insect was never known to be a cereal pest. This confirms that it is a new pest of cereal crops that makes a threat to cereals in North Tunisia.

Table 1.– Date and number of emerged *C. perplexella* adults under controlled and ambient conditions.

Rearing conditions	Date of emerged adults	Number of emerged adults
Controlled	17/02	1
	14/03	1
	15/03	2
	25/03	2
	27/03	2
	28/03	1
	05/04	1
Ambient	03/04	1
	10/04	1
	24/04	1

Eggs: We are obtained from reared adults with a 15% honey water solution in a separate box, where they were deposited on the extremity of a paper placed in a separate box. Eggs are fairly small, elongated, with a yellow-orange color (Fig. 3f). As preliminary observation and under controlled conditions, eggs required 15.83 days of incubation (Table 2).

Table 2.– incubation period of *C. perplexella* eggs.

Laying date	Hatching date	Incubation period
30/03	14/04	16
01/04	17/04	16
02/04	19/04	17
03/04	19/04	16
07/04	22/04	15
09/04	24/04	15
		Mean=15.83

The originality of this present work is resumed in three points: (i) the new detection of a *Coleophora* species in Tunisian entomofauna, (ii) the determination of its host-plant and (iii) its classification as a cereal pest. In fact, *Coleophora perplexella* Toll 1960 was described from specimens collected in Spain and Portugal and detailed by BALDIZZONE (2016). BALDIZZONE *et al.* (2006) cited its existence in Sardinia, Algeria and Morocco without the identification of the host-plant. Three years later, TAUTEL & NEL (2009) reported its presence in Toulon (France). The next year, NEL (2010) added that *C. perplexella* feeds on leaves of *Hordeum murinum* L. and *Bromus sterilis* L. (Poaceae).

To the best of our knowledge, there is just the work of BALDIZZONE (2016) who has described the adult and larvae of *C. perlexlella*. However, no additional works were performed on its biology. In this study we have presented a description of the nymph and eggs and their duration of pupation and incubation, respectively.

Our study contributed to the identification of new pest cereal insect in Tunisia making havoc on oat and barley plots in specific locations. In this work we have described the biology, illustrated the different pest stage and determined the length of case, caterpillar and wingspan adult and the eggs

incubation period. In the future, it will be necessary to perform deep studies of the pest biological and ecological particularities in the order to establish a control strategy. We propose, also, to study its geographic distribution in relation with its economic impact. Moreover, it will be needed to find out its invasion pathways in Tunisia, to include the effect of different agronomic scenario and bioclimatic zone on its kinetic and abundance.

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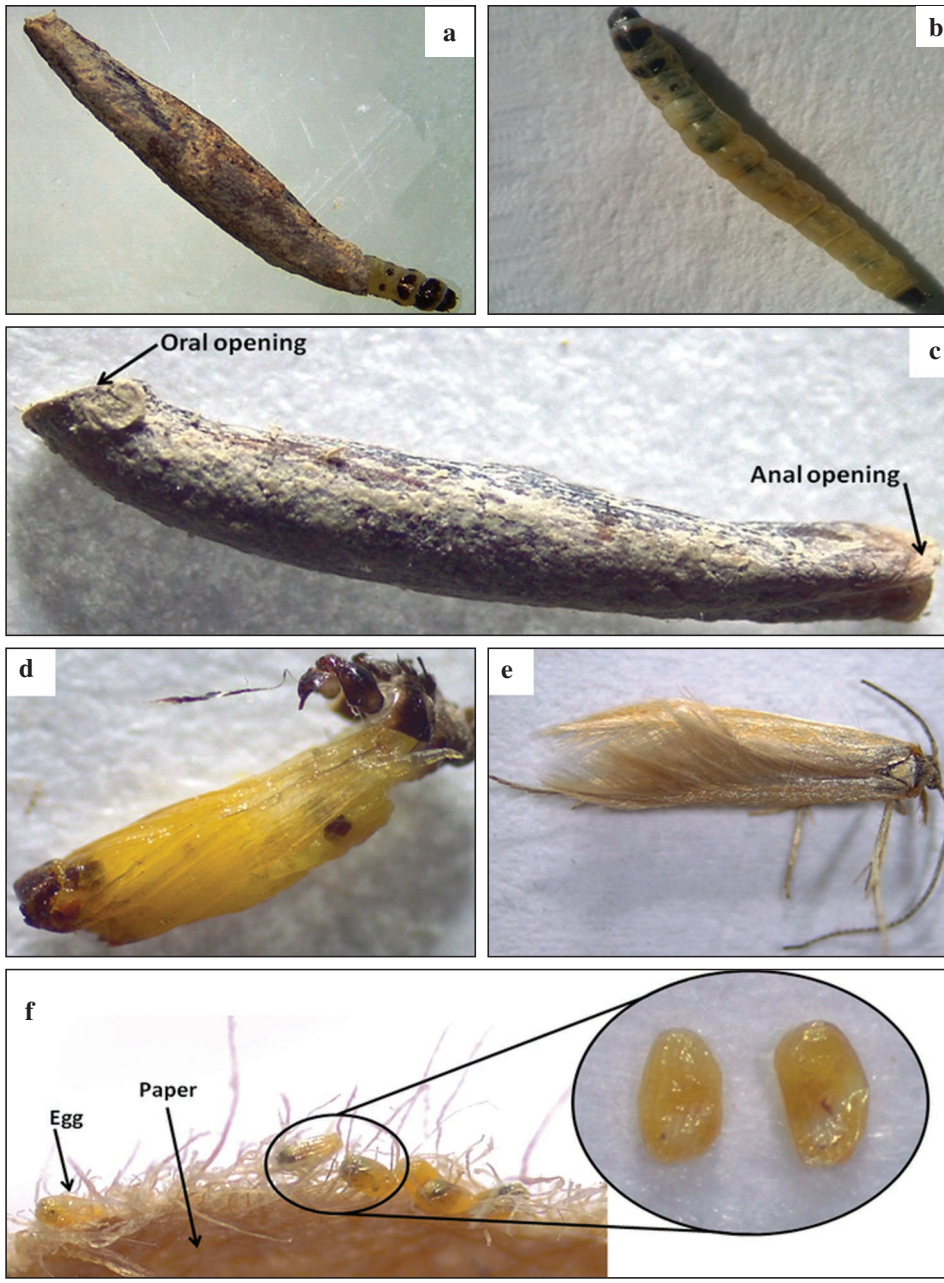

Figure 3.– Different biological stages of *Coleophora perlexella*: **a.** Larva protected within case, **b.** *Coleophora* caterpillar removed from its case, **c.** Larval case of collected insects, **d.** *Coleophora* nymph, **e.** Emerged adult, **f.** Eggs of *Coleophora perlexella.*